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NUTRITION REVIEWS

VOL. 8 APRIL, 1950 No. 4

CONTROL IN RESEARCH IN HUMAN NUTRITION

Among the factors which have confused the issue in nutritional and metabolic experiments in man must be named the superficial or inadequate concept of control and normal. A study of many reports of researches in human nutrition reveals that the controls are oftentimes fragmentary or forgotten. The whole weight of interpretation thus may resolve itself into the most patent empiricism. This coupled with the fact that relatively few clinicians have spent the time and effort necessary to arrive at competence, let alone excellence, in this most difficult medical discipline, helps explain the divergent separation on the spectrum of opinion between the optimists who see vitamin panacea everywhere and the skeptical iconoclasts who deny any virtue at all.

A brief historical note is apropos. In 1795–96, Giuseppi Cerri of Milan, Italy carried out what is probably one of the first carefully planned experiments in human nutrition. Convinced that there was some relation of food to the high incidence of pellagra among the countryfolk, he selected 10 pellagrins and for two years had them eat the diet of the townspeople. On this regimen no signs of pellagra developed, while in the untreated controls the incidence was high. The following year, allowed to revert to their polenta diet, pellagra came back as usual. The scientific pattern of this experiment with its exquisite simplicity has not been duplicated often until recently.

The astute clinical sense of Lind led to his careful observations on the cause and prevention of ship scurvy which enabled British seafarers to carve out a mighty empire. A similar clinical discovery by Takaki, elucidating the role of polished rice in producing beriberi, which riddled the ranks of the Japanese Navy, had profound though unforeseen consequences for the Western World. Goldberger, applying the science of an epidemiologist, cast light on the pathogenic background of pellagra by a series of observations culminating in production of a deficiency syndrome in previously unaffected persons.

During the last two decades rapid progress of discovery in the field of the B-complex vitamins by a brilliant combination of organic chemists, animal nutritionists, bacteriologists, and enzymologists, has provided clinicians with a wealth of new compounds for assay in malnourished persons. Let us examine some of the complexities of the problems as they relate to the B-complex vitamin family in human nutrition which may account for the uncertainties which beset us, and the confusion regarding the experimental control.

Mixed deficiencies: Since natural food sources tend to be rich or poor in all vitamin B-complex factors, it is rare for the clinical syndromes resulting from low supply to depend exclusively on single-factor depletion. At least there is wide overlapping, and it is not possible for diet history or clinical evidence to assess the exact role of the several factors concerned.

The variable human substratum: There is ample reason to suppose that individual response to precisely similar food depletion varies over a range from slight to severe. This distribution curve is influenced obviously by factors of age, growth, sex, size, pregnancy, lactation, menstruation, and physical activity, and in all probability by more subtle genetic, racial, and geographic influences. In the clinic we can scarcely approach the pure animal strain, fixed environment, measured food intake, and litter mate or inanition control of the animal nutritionist.

The variable past dietary experience: The

fallibility of memory concerning previous dietary experience presents difficulty in appraisal both qualitative and quantitative in type. But this uncertainty is compounded by the variability of vitamin and mineral content of a specific item of natural food in terms of its own genetic type, the seasonal, regional, and meteorologic situation attending its production, the many influences of harvesting, processing, preserving, storage, transportation, marketing, preparation, cooking, serving, and eating. It is indeed remarkable that clinical patterns of deficiency emerge with any consistency at all.

The nonspecificity of minor deviations from the normal: While there is general recognition and agreement upon the advanced or developed abnormalities which characterize rickets, scurvy, beriberi, and pellagra, minor changes of a similar type may be induced by a variety of non-nutritional causes. Unfortunately, simple chemical tests have not clarified the puzzle.

Unknown factors in the controlled experiment: Until it was pointed out by T. D. Spies (*Am. J. Med. Sci.* **184,** *837 (1932)*) that the lesions of pellagra might disappear while the patient was hospitalized even while eating a "pellagra-producing" diet it was not recognized that the upset balance between demand and supply could be rectified by reducing need as well as by enhancing supply. Thus any agent given at a time when this nonspecific effect of rest was causing improvement, was likely to be credited with specific effects. A great number of supposed remedies for pellagra had their origin in such misinterpretation.

Biosynthesis: It is not known to what extent the fabrication of vitamins from simple compounds by intestinal organisms can supply man's nutritional needs, but they have some importance.

Indirect effects of test substances: It is probable that certain vitamins which have effects on alimentary canal function, by reducing diarrhea, promoting biosynthesis, or facilitating absorption may permit some other vitamin to produce a clear effect which is mistakenly attributed to the test substance (W. B. Bean and T. D. Spies, *J. Am. Med. Assn.* **115,** *1078 (1940)*).

Lag in response: If a specific response takes weeks or months to occur it may be overlooked or therapy may be discontinued too early to observe it.

The humpty-dumpty situation: In conditions such as nutritional neuropathy when the nerve cell has died, no amount of therapy with the deficient substances will restore it. This may be misinterpreted as suggesting no relationship.

The problem of the target or effector mechanism: It would be a matter for surprise if all actively metabolizing cells did not have some need for all essential nutrients. In the case of specific types of cells such as nerves, their dysfunction can give rise to clinical difficulty only in rigidly stereotyped patterns. Presumably, therefore, several different deficiencies might produce the same or similar effects.

The problem of the single effect from multiple compounds: The work of W. A. Krehl, J. de la Huerga, and C. A. Elvehjem (*J. Biol. Chem.* **164,** *551 (1946)*) on the relation of tryptophan and niacin in animals, and the demonstration by R. W. Vilter, J. F. Mueller, and W. B. Bean (*J. Lab. Clin. Med.* **34,** *409 (1949)*) that tryptophan will relieve human pellagra illustrates another type of interrelation which may help clear up certain discrepancies.

Conditioning influences: It is still a matter for conjecture why such mechanical defects as intestinal shunts or anastomoses may be followed by pellagra, beriberi, macrocytic anemia, microcytic anemia, or reasonable health in apparently similar instances (W. B. Bean, T. D. Spies, and M. A. Blankenhorn, *Medicine* **23,** *1 (1944)*).

The role of vitamins as placebos: It must be recognized that conscious and unconscious factors may influence results of therapy and especially the interpretation of results. Experiments should always be set

up so the measurers of effect know nothing of who is getting placebo and who test compound.

Psychologic factors: Since the hospital has lost much of its terror, and famous clinics have done famous cures, one should try to assay individual emotional responses. Where so much is subjective (W. B. Bean, *J. Applied Physiol.* **1,** *458 (1948)*) the psychosomatic factors should be investigated and controlled.

This list could be continued but it illustrates sufficiently the complexity of the nutritional test and the matter of control in human beings. In speculating upon an ideal setup to study such matters, one envisions a large group of subjects, homogenous as to genetic factors, on controlled diets handled by a large metabolic team doing balance studies, varying single or multiple factors at will against a known background of past dietary experience, controlling energy output at will, measuring chemical and clinical changes, controlling biosynthesis, subjective influences, conditioning disease, and a host of other variables, or measuring those which are beyond control. Such a plan is utopian, but it is well to keep in mind such variables as those outlined to try to bring into line the discrepancies which characterize so much of the clinical literature on nutrition. With these variables in mind, and the critical accumulation of data which the natural history of man and disease provide, the science of nutrition, with its promise of better health for man, will eventually realize its highest goal.

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NEWER CONCEPTS IN ENERGY RELATIONS

The separate science of nutrition gained its early impetus through the studies of Carl Voit, Graham Lusk, and other pioneers in the field of energy metabolism. In a gross way it was recognized that energy was obtained by oxidation of foodstuffs, and this earliest concept has led to studies of biologic oxidation and the special processes by which burning of foods may yield energy for production of useful work and the synthesis of protoplasm.

Studies of the past several years have led to the concept that many of the individual oxidative (energy-yielding) reactions in metabolism are directed toward production of a common form of chemical energy in the nature of special molecular bonds involving phosphorus. Although several compounds are known which contain this special (energy-rich) form of phosphorus, the compound adenosinetriphosphate (ATP) appears to be the central one involved. Formation of energy-rich phosphate compounds and their utilization for vital processes have been reviewed by F. Lipmann (*Advances in Enzymology* **1,** *99 (1941); Nutrition Reviews* **6,** *198 (1948)*) and the purpose of this review is to consider only some recent contributions to the synthesis of ATP from the oxidation of foodstuffs and their metabolic intermediates.

With the thorough studies of glycolysis (lactic acid formation from glucose) it became apparent that ATP was formed by a transfer of energy-rich phosphate from compounds such as diphosphoglycerate and phospho-(enol)-pyruvate which themselves arise from energy-yielding reactions of glycolysis. It was also recognized that these anaerobic reactions could supply only a small part of the ATP synthesis which occurs in the total oxidative breakdown of the glucose molecule. Thus, in one of the early studies of ATP synthesis from aerobic metabolism (oxidative phosphorylation), S. Ochoa (*J. Biol. Chem.* **151,** *493 (1943)*) estimated that approximately three energy-rich phosphate bonds are formed for each

atom of oxygen utilized in the oxidation of pyruvate (P:O ratio of 3). Thus in the oxidation of the 2 moles of pyruvate from 1 mole of glucose, some 30 moles of energy-rich phosphate might be formed in contrast to the net formation of 2 moles of ATP formed during anaerobic glycolysis.

Since it is well known that pyruvate is oxidized through the members of the Krebs tricarboxylic acid cycle, it has been presumed that many of the reactions in this cycle yield energy-rich phosphate. It has been suggested that the ATP developed from these reactions might arise either from phosphorylated intermediates analogous to the intermediates in glycolysis, or during the passage of electrons derived from oxidation through the well-known chain of oxidation catalysts (pyridine nucleotides, flavoproteins, cytochromes).

Recent studies of aerobic phosphorylation and ATP synthesis have shed considerable light on the mechanism by which the energy locked in the foodstuffs and their intermediates is made available for useful work. In the first paper of a series of investigations, M. Friedkin and A. L. Lehninger (*J. Biol. Chem.* **178,** *611* (*1949*)) have demonstrated that inorganic phosphate is esterified and ATP is formed during the oxidation of reduced diphosphopyridine nucleotide (DPN-H_2), a coenzyme and member of the electron-transport system of oxidation catalysts. This was accomplished using phosphate containing P^{32} in a liver-tissue system containing essential additions of Mg^{++}, cytochrome-c, and ATP. Control experiments involving DPN instead of the reduced DPN-H_2, experiments conducted under anaerobic conditions, and others in which cytochrome-c was omitted, seem to leave little doubt that formation of ATP accompanies the passage of electrons from reduced DPN to oxygen. It was impossible to determine whether the ATP formation was connected more specifically with the steps involved from DPN-H_2 to cytochrome-c, or from the latter to oxygen.

In a further paper, A. L. Lehninger (*J. Biol. Chem.* **178,** *625* (*1949*)) has reported studies on oxidative phosphorylation during the oxidation of beta-hydroxybutyrate to acetoacetate. This oxidation, dependent on DPN as a coenzyme, was particularly convenient for use with the same rat liver preparation previously employed, since no further complicating metabolism of the acetoacetate occurred. Here again an esterification of inorganic phosphate and ATP formation was found, which on thermodynamic grounds and by direct experiment, was directly referable to the process of electron passage from DPN to oxygen. The sensitive nature of the phosphorylation process was illustrated. Thus a relatively short incubation of the enzyme preparation or the addition of arsenite, 2,4 dinitrophenol, p-nitrophenol, Ca^{++}, or SO_4^{--} resulted in failure of phosphorylation without affecting the rate of oxidation of beta-hydroxybutyrate to acetoacetate. The effect of these agents has been commonly referred to as an "uncoupling" of oxidation and phosphorylation and has been noted by other investigators. As in the previous study it was difficult to associate oxidative phosphorylation with any particular phase of the electron-transport mechanism. Some interesting discussion was offered as to whether the "uncoupling" due to the state of the enzyme preparation might be referable to different pathways of electron transport or to dual functions of oxidation catalysts.

A paper by J. P. Hummel and O. Lindberg (*J. Biol. Chem.* **180,** *1* (*1949*)) is of interest in localizing the oxidation catalyst concerned with phosphorylation in the electron-transport system. The work of these investigators is also an excellent illustration of the use of two modern and powerful tools of biochemistry, namely, paper partition chromatography (*Nutrition Reviews* **7,** *195* (*1949*)) and isotope tracing. For their purpose a rabbit-liver homogenate was vigorously aerated with glutamate and inorganic phosphate containing P^{32}. A sample of the

trichloroacetic acid filtrate was used for studies on ATP, and the remainder was appropriately extracted to obtain a fraction containing the flavin and pyridine nucleotides. Such samples were subjected to paper chromatography utilizing five different solvent systems. The compounds that could be satisfactorily separated by one or another of these solvent systems were adenylic acid, adenosinetriphosphate (ATP), diphosphopyridine nucleotide (DPN), flavin mononucleotide, and flavin dinucleotide. Methods were also developed to estimate the amounts of the different components present on the paper. The filter-paper strip containing these components could also be scanned for radioactivity, through a small window, in conjunction with the counting device to determine the distribution of P^{32} on the paper.

With these measurements on the chromatograms produced with the various solvents, conclusions could be drawn on the association of P^{32} with the different components listed above. The P^{32} was found with the ATP fraction, as would be expected from the earlier reports, but also with the flavin-adenine dinucleotide component, and in such a concentration as to indicate that such flavins are engaged in aerobic phosphorylation. The DPN or flavin-adenine mononucleotide fractions did not contain radioactivity.

These results, therefore, indicate that the high-energy phosphate bond might reside temporarily in flavin catalysts, before being transferred to an adenine nucleotide for ATP synthesis. The nonparticipation of DPN in this process is contrary to earlier suggestions, although it is not impossible that it could participate in other aerobic phosphorylation systems. Since there is a large difference between the oxidation-reduction potentials of the flavins and cytochrome oxidase, it is possible that another oxidation catalyst, perhaps a cytochrome component, is also involved directly in aerobic phosphorylation.

METABOLIC STUDIES OF CARDIOVASCULAR TISSUE *IN VITRO*

Although cardiovascular disease and cancer are both leading causes of death in this country, study of the basic biochemical processes of the heart and its associated vascular tissues has not received the same attention by researchers in the medical sciences as has study of these processes in tumor tissue. Increasing interest in the biochemistry of the cardiovascular system is apparent, however, from the recent publication of a number of investigations of the metabolic behavior of cardiac muscle and the large blood vessels from animals *in vitro*. Since both congestive heart failure and development of atherosclerosis may be aggravated in patients with marked metabolic disturbances, information about the metabolism of cardiovascular tissue in different states of nutrition and endocrine balance is much needed.

Several studies of the respiration and utilization of substrate by slices of cardiac muscle from normal animals have appeared. R. E. Olson, O. H. Pearson, O. N. Miller, and F. J. Stare (*J. Biol. Chem.* **175,** *489 (1948)*) studied the fate of pyruvate in slices of heart ventricle from healthy rats and ducks and found that disappearance of added pyruvate could be accounted for partially by reduction to lactate and partially by oxidation under aerobic conditions. At 5 millimols per liter, conversion of pyruvate to lactate amounted to 10 to 20 per cent of the total disappearance, and conversion of pyruvate to nonlactate products always exceeded the amount requiring oxidation in order to produce the stimulation of respiration observed.

By adding pyruvate labeled either in the carboxyl or carbonyl groups with C^{14} to normal heart slices from ducks, R. E. Olson and F. J. Stare (*Federation Proceedings* **8,** *391 (1949)*) were able to demonstrate that 40 per cent of the pyruvate which disap-

peared to nonlactate products was metabolized without loss of carbon dioxide, presumably in anabolic reactions, and that the total contribution of pyruvate combustion to the oxygen consumption of the slice at 5 millimols per liter amounted to 50 per cent of the Q_{O_2}. In experiments with carbonyl-labeled pyruvate as substrate for rat ventricle slices, O. H. Pearson, A. B. Hastings, and H. Bunting (*Am. J. Physiol.* **158,** *251 (1949)*) found that oxidation to carbon dioxide accounted for 85 per cent of the pyruvate which disappeared to nonlactate products and for 77 per cent of the total oxygen consumption of the slice. Both Olson *et al.* and Pearson *et al.* found that carboxyl-labeled acetate was oxidized less rapidly than carbonyl-labeled pyruvate in slices of ventricle.

The effects of anoxia, variations in slice thickness, ionic composition of the medium, and addition of different substrates upon the respiration of heart muscle slices from rats have been studied not only by Pearson, Hastings, and Bunting (*loc. cit.*) but by J. L. Webb, P. R. Saunders, and C. H. Thienes (*Arch. Biochem.* **22,** *444, 451 (1949)*). These investigations are in agreement in so far as it was found that anoxia markedly depressed respiration of cardiac muscle slices, that optimum slice thickness was 0.4 to 0.5 mm., that relatively large variation in the ionic composition of an isotonic medium could be made without effect upon the respiration of heart slices, and that substrates such as acetate, pyruvate, lactate, citrate, and other intermediates of the Krebs tricarboxylic acid cycle, particularly succinate, resulted in marked stimulation of respiration.

In a third paper, J. L. Webb, P. R. Saunders, and C. H. Thienes (*Arch. Biochem.* **22,** *458 (1949)*) report that malonate, fluoride, iodoacetate, cyanide, phlorhizin, and pyrophosphate inhibit the endogenous respiration of rat heart slices. O. N. Miller, R. E. Olson, and F. J. Stare (*Federation Proceedings* **7,** *174 (1948)*) showed that reactivity of normal heart slices toward substrate, as evidenced by total disappearance and oxidation of pyruvate, varied with the time of addition of substrate and the length of period of incubation, and were able to plot the metabolic decay of heart slices *in vitro* as a function of time.

The effects of various vitamin and hormone deficiencies upon the metabolism of cardiac muscle *in vitro* have also been reported. Olson, Pearson, Miller, and Stare (*loc. cit*) found that thiamine deficiency in rats and ducks resulted in a decrease in the ability of cardiac muscle slices to oxidize pyruvate which could be corrected by the *in vitro* addition of thiamine. Large decreases in heart thiamine were required before impairment of pyruvate utilization occurred, but below levels of 2.5 micrograms of thiamine per gram fresh weight of ventricle, the rate of conversion of pyruvate to nonlactate products was a function of the thiamine content. It was also observed that both the endogenous lactate formation and that occurring in the presence of added pyruvate were increased more by inanition than by thiamine deficiency.

In biotin-deficient ducklings, R. E. Olson, O. N. Miller, Y. J. Topper, and F. J. Stare (*J. Biol. Chem.* **175,** *503 (1948)*) observed that both the endogenous respiration of slices of auricle and ventricle, and that occurring in the presence of added pyruvate were reduced about 40 per cent below normal. Conversion of pyruvate to nonlactate products and production of $C^{14}O_2$ from radioactive carboxyl-labeled succinate were also reduced about 50 per cent in biotin-deficient ventricle. The *in vitro* addition of biotin to deficient heart slices had no effect upon the depressed metabolism, although injection of deficient ducklings with biotin restored their myocardial metabolism to normal in twenty-four hours. These observations are consistent with the present view of the role of biotin in oxalacetic acid metabolism (*Nutrition Reviews* **5,** *355 (1947)*)

and suggest, in addition, that the active form of the vitamin in tissues is a complex derivative.

In slices of ventricle from pantothenic acid-deficient ducklings, Olson and Stare (*loc. cit.*) found that the rate of oxidation of radioactive pyruvate was within normal limits despite marked decreases in coenzyme A content (Olson and N. O. Kaplan, *J. Biol. Chem.* **175,** *515* (*1948*)) and a 25 per cent decrease in pyruvate disappearance. Homogenates of deficient ventricle, however, were markedly depressed in their ability to oxidize labeled pyruvate or to synthesize citrate from pyruvate and fumarate (Olson, E. G. Hirsch, H. Richards, and Stare, *Arch. Biochem.* **22,** *480* (*1949*)). These observations are of interest, not only in connection with the mode of action of pantothenic acid and coenzyme A in pyruvate utilization (J. R. Stern and S. Ochoa, *J. Biol. Chem.* **179,** *491* (*1949*)), but also as examples of the degree to which intact cells must be depleted of certain coenzymes before recognizable biochemical lesions occur.

In a study of folic acid deficiency in the duckling, O. N. Miller, R. E. Olson, and F. J. Stare (*Federation Proceedings* **8,** *390* (*1949*)) found decreases in the respiration of erythrocytes but not of cardiac muscle slices when incubated with pyruvate. Decreases in the total utilization of pyruvate by slices of ventricle, however, were observed and suggest that this deficiency interferes either directly or indirectly with the anabolism of pyruvate.

A study of the metabolism of cardiac muscle slices from rats made diabetic with alloxan by O. H. Pearson, C. K. Hsieh, C. H. Dutoit, and A. B. Hastings (*Am. J. Physiol.* **158,** *261* (*1949*)) has revealed that both the disappearance of radioactive carbonyl-labeled pyruvate and its oxidation to $C^{14}O_2$ are decreased in ventricle from diabetic rats and not increased by the *in vitro* addition of insulin. The oxidation of carboxyl-labeled acetate, however, was not decreased below normal in heart slices from diabetic rats.

Since knowledge regarding the metabolism of the blood vessels may be as important for the study of atherosclerosis as are basic studies in cardiac muscle metabolism for the understanding of certain types of cardiac failure, the paper by F. N. Briggs, S. Chernick, and I. L. Chaikoff (*J. Biol. Chem.* **179,** *103* (*1949*)) is of special interest. In this study thoracic and abdominal aorta and the inferior vena cava of rats were freed of adhering connective tissue and fat, slit longitudinally, and pooled to provide sufficient amounts of tissue for *in vitro* studies of oxygen consumption and substrate utilization by the technic of Warburg. It was found that aortic tissue respired at a rate equal to one-tenth that of liver or heart muscle, while the oxygen consumption of the vena cava was about one-fifteenth this value. No significant differences were found in the respiration of the blood vessels of "young" rats 1 to 2 months of age and "old" rats 14 to 18 months of age. As in the case of other aerobic tissues, addition of substrates such as succinate, pyruvate, lactate, alpha-ketoglutarate, and citrate to aorta resulted in increases in respiration while addition of malonate, fluoride, azide, and iodoacetate caused inhibitions.

A significant increase (25 to 38 per cent) in the oxygen consumption of thoracic aorta tissue was found in animals fed desiccated thyroid, and a decrease (19 to 28 per cent) was found in animals fed propylthiouracil. These investigators point out that this is of interest since it has been shown that development of atherosclerosis in rabbits fed cholesterol is prevented by administration of desiccated thyroid (I. H. Page, *Biol. Symposia* **XI,** *43* (*1945*)).

Lipid synthesis in aortic tissue from the rat was studied with the aid of radioactive carbon-labeled acetate and inorganic phosphate labeled with P^{32} by S. Chernick, P. A. Srere, and I. L. Chaikoff (*J. Biol. Chem.* **179,** *113* (*1949*)). Incorporation of labeled ace-

tate ($C^{14}H_3C^{14}OOH$) into fatty acids of the aorta during a three-hour incubation period was one-third to one-fifth as rapid as in rat liver slices. When inorganic phosphate labeled with P^{32} was added, the rate of incorporation of the radioactive phosphorus into aortic phospholipid was one-fifth to one-seventh that of rat liver slices.

Although certain current hypotheses of the etiology of atherosclerosis (*Nutrition Reviews* **8,** *41, 61, 74 (1950)*) assign a passive role to the artery and emphasize the importance of intimal injury or the state and amount of the blood lipids, it is possible, as these workers point out, that arterial tissue may play a more active role in the pathogenesis of this type of vascular disease by altering its own metabolism.

GALACTOSE UTILIZATION

Galactose excretion in the urine of rats following ingestion of lactose was observed as early as 1938 by E. J. Schantz, C. A. Elvehjem, and E. B. Hart (*J. Biol. Chem.* **122,** *381 (1938)*). When mineralized skim milk was fed by these investigators, as much as 35 per cent of the ingested galactose was excreted. When whole milk was fed, or when skim milk was supplemented with one of a series of fats at 4 per cent, utilization of galactose was found to be perfect.

Later investigators using different procedures have had difficulty, or have found it impossible, to demonstrate a relation between fat ingestion and the efficiency of galactose utilization by the rat. By way of contrast to the above observations, some evidence has been obtained by the "single-food-choice technique" that galactose had a marked beneficial effect on utilization of vegetable fats when galactose and the fat source were fed together as the sole sources of nutrients (*Nutrition Reviews* **7,** *148 (1949)*). In these trials, the amount of galactose chosen by the rats was very low in comparison to the levels often fed in purified diets or occurring in skim milk.

The metabolism of galactose is not at all well understood at present and a considerable amount of fundamental knowledge is needed to determine the various phases concerning how it is utilized and why high levels produce severe toxic manifestations. In rare cases, it has been demonstrated that infants have a high blood sugar, about half of which is galactose, along with an extensive galactosuria (*Nutrition Reviews* **4,** *204 (1946)*). In the aged, the rate of absorption of galactose given orally is significantly lower than in younger individuals (*Ibid.* **4,** *172 (1946)*).

As a contribution to the knowledge of galactose utilization, V. H. Barki *et al.* (*J. Biol. Chem.* **181,** *565 (1949)*) have made a series of studies concerning the factors which influence the utilization and excretion of galactose by the rat. Throughout these experiments, total reducing substances in the urine were determined by a modification of the micromethod of M. Somogyi (*Ibid.* **160,** *69 (1945)*); after the fermentable sugars were removed by incubation in the presence of baker's yeast, the reducing substances were determined again on the filtrate. The value obtained in the second determination represented the amount of nonfermentable-reducing substances in the original urine samples, one of which would be galactose after periods of galactose or lactose feeding.

To determine the amount of reducing substances, other than galactose, in rat urine, samples were collected from rats fed a diet devoid of galactose and lactose. The average daily excretion of nongalactose-reducing substances was found to be 15.7 mg. per 100 g. of body weight. Thus, the calculated value for urinary galactose was determined by subtracting the value for nongalactose-reducing substances from the value for total nonfermentable-reducing substances.

Four experiments were conducted, three with purified diets and one with milk. Rats weighing 120 g. and housed in metabolism cages were used in the investigations. Daily food consumption was determined and the urine was collected under toluene. In the first experiment, galactose was administered in known amounts by stomach tube thrice daily for three or four days. The remaining dietary requirements for the three groups of rats were provided by a diet mixture composed of: (1) casein, salts, and butterfat; (2) casein, salts, and sucrose; or (3) casein at a high level and salts. Under these circumstances, there was an extremely high rate of galactose excretion in the urine. The percentage of galactose excreted increased rapidly with increases in oral administration of galactose. For example, in rats fed the casein, salts, and sucrose mixture, the galactose excretion varied from about 37 per cent when 0.6 g. was tube-fed daily per 100 g. of body weight, to almost 70 per cent when 2.7 g. were tube-fed daily per 100 g. of body weight. These were the highest rates of galactose excretion noted throughout the experiments. At all levels of galactose ingestion, the presence of butterfat in the diet resulted in a slightly lower rate of galactose excretion. Presence of a high level of casein was not beneficial at low levels of galactose ingestion but, at higher levels, appeared to be slightly helpful in reducing excretion of galactose.

In the second experiment, the same design was followed with the single exception that galactose was incorporated into the three diet mixtures at appropriate levels to provide similar degrees of galactose consumption as in the above experiment. In this way, the galactose was ingested at a much slower rate over a greater period of the day than at the three specific tube-feeding times. The amounts of galactose excreted by the rats in this experiment were considerably less than in the first project, especially at low levels of galactose intake. Again, the diet with sucrose and galactose in it permitted the highest rates of galactose excretion. At an intake of about 0.7 g. per day per 100 g. of body weight, approximately 15 per cent of the galactose was excreted, while at an intake of 3 g. per day per 100 g. of body weight, a little over 60 per cent of the ingested galactose was excreted in the urine. A high level of casein was not particularly helpful at any level and may have resulted in an increased galactosuria at the highest level of galactose intake. At all levels, the simultaneous ingestion of butterfat resulted in slight decreases in the percentage of galactose excreted.

In the third experiment with purified diets, lactose was incorporated in the same three diet mixtures as a source of galactose. The amount of lactose to be introduced into these rations was calculated on the basis of the galactose it would supply upon hydrolysis in the body to galactose and glucose. The toxicity of lactose evidenced in this experiment by severe diarrhea prevented trials at as high levels of galactose ingestion as those used in the first two experiments. Ingestion of lactose instead of galactose directly resulted in an extremely low-galactose excretion when compared with the results of the two earlier experiments. Again the animals which received butterfat along with the galactose-containing diet had a slightly lower degree of galactosuria than their counterparts on the diets with sucrose or with a high-casein content.

The fourth experiment was conducted with whole milk, skim milk, and skim milk to which cerelose had been added. The volumes of these three fluids were adjusted so that the lactose concentrations per unit volume were identical from one to the other. The rats fed whole milk did not excrete any galactose, but those fed skim milk alone or skim milk plus cerelose excreted low and similar amounts of galactose.

The data from these experiments indicate clearly that the major factors controlling galactosuria are: (1) the quantity of galactose ingested in proportion to body weight,

and (2) the rate at which galactose enters the blood stream. A high-galactose excretion occurred when this sugar was given in large doses by stomach tube; an intermediate degree of galactosuria was observed when the same amount of galactose was ingested slowly over a twenty-four-hour period; galactose excretion was very low when the galactose was slowly liberated during normal hydrolysis of lactose.

In all cases, administration of fat along with the galactose or lactose resulted in a lesser degree of galactosuria. However, this reduction was not considered by the investigators to be sufficiently large to necessitate any mechanism other than the known role of fat in slowing gastric emptying and thus delaying absorption of galactose from the intestine. The presence of a higher amount of casein than is needed to maintain positive nitrogen balance, or the presence of sucrose, had no comparable effect on the excretion of galactose in contrast to the effect with fat.

ABSENCE OF INOSITOL FROM ALPHA-AMYLASE

Several papers (*Nutrition Reviews* **7,** *120 (1949)*) have suggested an antagonistic relation between inositol and gamma-hexachlorocyclohexane (gammexane, 666). In one of the most striking of these, a possible coenzymatic role of inositol in the action of amylase was suggested by the observation that purified alpha-amylase of pancreas contained considerable quantities of inositol, that its amylolytic activity was inhibited by incubation with gamma-hexachlorocyclohexane, and that inactivation was prevented by simultaneous incubation with gamma-hexachlorocyclohexane *and* inositol (R. L. Lane and R. J. Williams, *Arch. Biochem.* **19,** *329 (1948)*; *Nutrition Reviews, loc. cit.*).

This problem has been further investigated by E. H. Fischer and P. Bernfeld (*Helvet. chim. acta* **31,** *1146 (1949)*). These authors had worked out procedures for obtaining alpha-amylase in highly active, crystalline form from saliva and from pork pancreas. Hydrolysates of these crystalline preparations showed no detectable inositol when assayed with the "inositol-less" mutant of *Neurospora crassa*. The levels assayed were sufficiently high so that it was certain that less than 1 molecule of inositol was present for every 20 molecules of enzyme. Thus inositol is not a component of pure alpha-amylase. The authors cited unpublished data to show that inositol did accompany pancreatic amylase through preliminary purification steps, and attribute the presence of inositol in the preparation assayed by Lane and Williams to incomplete purification. Experiments with gamma-hexachlorocyclohexane and alpha-amylase were repeated. No inactivation of amylase due to this substance could be found. Similarly, no activation of their amylase preparations followed incubation with inositol. It must be concluded, therefore, that inositol plays no essential role in the activity of this enzyme.

The concept that gamma-hexachlorocyclohexane owes its toxic effects to displacement of inositol from one or more essential loci *in vivo* was advanced at a time when the spacial configuration of this substance was thought to be identical with that of meso-inositol. Since that time, G. W. Van Vloten and co-workers (*Nature* **162,** *771 (1948)*) have shown by physical methods that the spacial configuration of gamma-hexachlorocyclohexane and meso-inositol do not correspond to one another. For this reason, it may not be surprising that the toxic effects of this insecticide for most organisms do not appear to be alleviated by inositol, although the latter substance does appear to protect other organisms against small amounts of the compound (W. H. Schopfer, T. Posternak, and M. L. Boss, *Schweiz. Z. Path. u. Bakt.* **10,** *443 (1947)*; P. Chaix, L. Lacroix, and C. Fromageot, *Biochim. et Biophys. Acta* **2,** *57 (1948); Nutrition Reviews, loc. cit.*).

ATHEROSCLEROSIS IN DIABETIC RABBITS

Atherosclerosis is a major complication which appears often in diabetics. Insulin and dietary control have not solved this problem. From the experimental standpoint, the interrelations between diabetes and atherosclerosis have been difficult to study since the animals most used in diabetes research are not ordinarily susceptible to atherosclerosis. On the other hand, the rabbit, in which atherosclerosis is easily developed, has been difficult to use in diabetes research.

A way out of this dilemma appeared when it was shown that rabbits could be made permanently diabetic with alloxan (J. S. Dunn, H. L. Sheehan, and N. G. B. McLetchie, *Lancet* **I,** *484 (1943)*). Such animals exhibited gross lipemia (C. C. Bailey and O. T. Bailey, *J. Am. Med. Assn.* **122,** *1165 (1943)*), presumably reflecting some hypercholesterolemia, a condition which might be expected to produce atherosclerosis in this species. However, atherosclerotic lesions were not observed in short-term experiments.

On closer examination (F. E. Kendall, W. Meyer, L. Lewis, and J. Victor, *Proc. Soc. Exp. Biol. Med.* **60,** *190 (1945)*; G. L. Duff and D. C. Wilson, unpublished data quoted in the article by Duff and G. C. McMillan, *J. Exp. Med.* **89,** *611 (1949)*), it was found that the lipemia in alloxan-diabetic rabbits was transient and inconstant. While the blood cholesterol was frequently elevated to about 350 mg. per cent, it almost always returned to normal in three to six weeks and remained so during the remainder of the animal's life despite the persistence of severe diabetes. Atherosclerosis did not develop in periods up to one year.

L. Swell, N. P. Goldstein, and C. R. Treadwell (*Endocrinology* **45,** *57 (1949)*) have studied the behavior of cholesterol and its esters in alloxan-diabetic rabbits with results similar to those mentioned above. In their experiments the peak total blood cholesterol values were found seven to ten days after induction of diabetes. These peak values ranged from 89 to 688 mg. per cent, an increase over control values of 92 to 1170 per cent. After this peak, the cholesterol values returned toward normal and had reached control values by the seventeenth day. Free cholesterol more or less paralleled total cholesterol. The changes in cholesterol esters were different. As the total cholesterol increased, the esters showed no consistent change so that a lowering of the ester to total cholesterol ratio occurred. This ratio returned to normal on about the twelfth postalloxan day.

After the diabetes had assumed a steady state (twenty-one to thirty-three days), the rabbits were given protamine-zinc insulin. By the tenth day of insulin administration, there was a definite increase, of moderate degree, in the total cholesterol in 5 of 6 rabbits which was paralleled by comparable increases in free cholesterol and by a decrease in the ester:total cholesterol ratio. These changes were similar to those occurring immediately after alloxan and also were transitory.

Since atherosclerosis had not appeared spontaneously in alloxan-diabetic rabbits, G. L. Duff and G. C. McMillan (*J. Exp. Med.* **89,** *611 (1949)*) studied the production of atherosclerosis by feeding excess cholesterol to rabbits already made diabetic with alloxan. Insulin was used to maintain the animals in the early periods after alloxan but was not used during cholesterol feeding. Control and diabetic rabbits received either dry powdered cholesterol in capsules or a solution in corn oil to yield daily intakes of 0.18 to 0.75 g. for fifty-two to ninety-one days. Cholesterol administration was begun only after the diabetes was well stabilized. Eighteen diabetic and 39 nondiabetic animals, the latter including 13 rabbits which had recovered from alloxan, were used in the study.

The lower dosages of cholesterol produced neither a hypercholesterolemia nor atherosclerosis in either control or diabetic rabbits. With the higher dosages, the control and

alloxan-recovered rabbits consistently showed hypercholesterolemia and severe atherosclerosis. Diabetic rabbits receiving equivalent amounts of cholesterol showed a striking resistance to the development of atheromatous lesions though their blood cholesterol, both free and esterified, was increased just as much as in control animals. Furthermore, diabetic rabbits showed much more severe gross lipemia of the serum than did control animals.

In view of this striking and completely unexpected finding, it was logical to speculate whether alloxan diabetes might favorably influence the course of atherosclerosis which had been induced in rabbits prior to administration of alloxan. Duff and McMillan (*loc. cit.*) tested this possibility in 32 rabbits. However, the results showed that alloxan diabetes had no influence on the rate or degree of retrogression of the atherosclerosis when control and experimental animals were compared. Likewise, the diabetic state had no influence on the rate or degree of disappearance of abnormal lipid deposits in the liver, spleen, and adrenal cortex.

Neither control nor diabetic rabbits showed gross morphologic evidence of retrogression of the aortic atherosclerosis in periods up to four months after cholesterol dosage was discontinued. However, it is of considerable interest that there was microscopic evidence of some disappearance of lipid deposits beginning about six weeks after the cholesterol was stopped. In the aorta, the gradual disappearance of lipid material was accompanied by a gradual increase in the number of fibroblastic cells. This observation gives some hope that atherosclerosis may be reversible to some extent when the abnormal diet is corrected.

The inhibitory effect of alloxan diabetes on the development of atherosclerosis, as observed in these experiments, would seem to indicate the existence of hitherto unrecognized factors which influence this process, although the nature of this factor or factors is completely obscure. Clearly, hypercholesterolemia is not the only factor since the alloxan-diabetic rabbits failed to develop severe atherosclerosis despite markedly increased blood cholesterol.

It may well be that separate factors control the deposition and resorption of lipids. This possibility is strengthened by the observation that alloxan diabetes failed to influence the regression of atherosclerosis although it inhibited its formation.

These experiments offer little possibility of direct application in human arterial disease. However, they do provide a basis for further experimentation designed to elucidate the nature of the various factors which govern the process of lipid deposition in the walls of arteries. A clear understanding of these factors would be of great importance in the understanding and treatment of human disease.

I. BASIC CONCEPTS IN ASSESSMENT OF HUMAN NUTRITURE

The clinical symptomatology of the fully developed classic nutritional deficiency diseases—starvation, night blindness, rickets, beriberi, pellagra, scurvy—is fairly well established. It is good to have these facts on the books. But the occasions on which such knowledge is useful to a nutritionist working in the United States, outside the relatively limited area of the South in which pellagra is encountered, are rather rare. What the medical examiner engaged in a nutrition survey looks for are the numerous, far less obvious stigmata of the "subclinical nutritional deficiencies."

Whereas the earlier investigators were prone to be enthusiastic about the specificity of these signs as indicators of nutritional deficiencies, further research counseled caution. The publication of the Food and Nutrition Board of the National Research Council

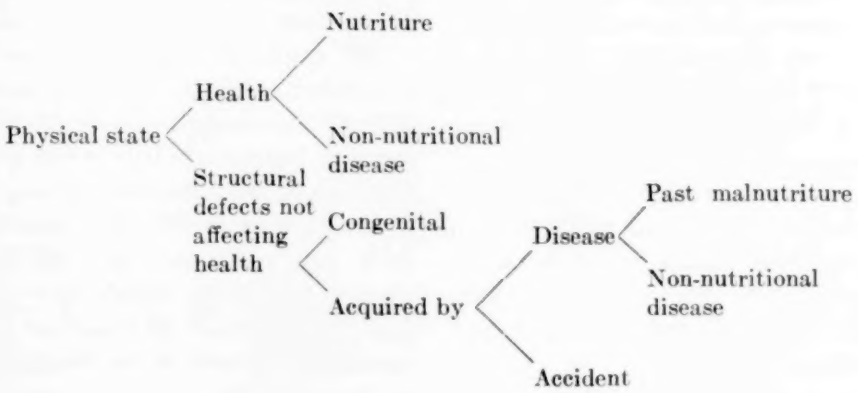
dealing with clinical diagnosis in nutrition surveys presents the view "that there is no such thing as a single pathognomonic sign, that interpretation of clinical findings in a given individual requires careful differential diagnosis including history, physical examination, and laboratory findings, and that in many instances a therapeutic diagnosis is necessary to settle clinical judgments" (*National Research Council Bull.* **117,** *83 (1949)*).

This passage is symptomatic of the growing emphasis on *critical* appraisal of the signs and measures of nutritional status. The enthusiasm of the earlier investigators, engaged in nutritional research and in nutrition surveys in particular, enthusiasm not always coupled with strict observance of the canons of scientific method, is giving way to a critical accumulation of facts and evaluation of the basic premises of assessing man's nutritional status. Such an evaluation was attempted by H. M. Sinclair (*Vitamins and Hormones* **6,** *101 (1948)*). The material will be reviewed in three articles. The first one deals with the general concepts while the second and third articles will be devoted to specific methods (dietary, chemical, functional, somatometric, and clinical).

"Nutriture" (*Nutrition Reviews* **4,** *287 (1946)*), the nutritional state or condition of the body, is the end result of a manifold metabolic activity, termed "nutrition." The nutrition of the cells of the body involves four categories of substances or nutriments: aliments, nutrients, oxygen, and water. The aliments are defined as nonspecific chemical compounds used by the body for production of energy needed for keeping the machinery of the body running and for external work. They may also be used for growth or repair. The aliments include carbohydrates, fats, and proteins. The nutrients are specific chemical substances, essential for the processes of body metabolism as well as for growth and repair. They include compounds of some elements (iron, magnesium), essential amino acids, essential fatty acids, provitamins, and vitamins. These substances either cannot be synthesized by the body at all or not in sufficient amounts and must be supplied, like the aliments, by food ingested by the organism.

Nutrition involves ingestion, digestion, absorption, transport in the blood, diffusion into extracellular fluid, and passage into the cells. To secure proper nutrition, the aliments and nutrients must be present in the cells in adequate concentration, together with water and oxygen, and removal of products of metabolism must be effective. Nutrition may be impaired by the presence of toxic substances in the cells and by the action of physical agents, such as rise in temperature.

In the nutritional literature there is much confusion as far as the meaning of nutriture, health, and physical state is concerned. Sinclair's definitions of these terms are of interest. The interrelations are indicated on the following diagram:



Good physical state is defined by absence of illness and of structural abnormalities (loss of one leg, cleft palate, rachitic deformities as residue of past malnutriture) not affecting health. Good health depends upon the presence of good nutriture and the absence of (non-nutritional) diseases, such as appendicitis. Nutriture is characterized in terms of three principal properties: quality, degree, and duration. The quality of nutriture (malnutriture) refers to the specific nutritional deficiencies resulting from the deficiency of a particular nutrient. The specificity is rarely, if ever, absolute. Thus deficiency of a specific nutrient (*e.g.*, thiamine) may result in caloric undernutrition and to the specific signs of the (thiamine) deficiency may be added such signs of deficiency of aliments as diminution of adipose tissue and atrophy of the testes.

Five degrees of nutriture are distinguished, with surfeit of aliments or nutrients and their lack as the two extremes. Death occurs at either end of the scale. The boundaries between the five degrees are fluid. On the upper end, the excess nutriture is due to excessive supply resulting in impaired function and structural abnormalities. Obesity resulting from an excessive intake of carbohydrates; ketosis in the case of excess ingestion of fat; brittle teeth due to oversupply of fluorine may be cited as examples of excess nutriture.

In the state of normal nutriture, body function and structure are unimpaired by nutrition and the body has reserves adequate for meeting ordinary demands. In poor nutriture there is no impairment of function or structure but the reserves are lowered below a safety zone and impairment occurs if the body is exposed to a physiologic stress (such as pregnancy and lactation). In latent malnutriture the undeveloped impairment may be detected by special tests but no clinical signs of deficiency are present. In clinical malnutriture there is a definite, marked impairment of function or structural changes in the tissues.

The duration of nutriture refers to the time during which the state existed. This concept should not be confused with the acuteness or chronicity of the nutritional process, indicated by the rate of change in nutriture, its rapidity, or slowness. The same amount of cumulative deficit will result in different states of malnutriture when the deficit is acute and when it is chronic.

In speaking of nutritional deficiency one should make clear whether one refers to (a) deficiency in the diet (inadequate ingestion); (b) decrease in the concentration of a nutriment in the cells of the tissues (decreased bodily reserves); (c) the resulting degree of alteration in the process of nutrition in the cells (impairment of metabolism); or (d) the changed degree of nutriture produced by the biochemical derangement (impairment of physiologic functions, structural alterations, microscopic and macroscopic).

It may be appropriate to conclude this first part of the review of Sinclair's stimulating paper by discussing his concept of normality. Obviously, impairment of function and disease must have their reference point in the "normal" function and in health. Sinclair distinguished four concepts of normality. Normality may be identified with an arbitrarily defined perfection. Thus some may describe the normal (*i.e.*, ideal) nutriture, as far as ascorbic acid is concerned, as the state of saturation in which the vitamin is spilled in the urine. In addition to the arbitrary character of such criteria, Sinclair noted that "ideal" nutritional state may be, from another angle, a handicap (*cf.* nutriture of a marathon runner and a football player).

The second definition identifies normality as freedom from signs or symptoms of active disease; the concept of normality defined in terms of frank pathology is too gross for the purposes of the nutritionist although it may be satisfactory for the clinician. Physiologically, man's physical condition is "normal" when the relatively steady state of the body is undisturbed and all functions can be adequately performed in the usual (customary) environment.

Finally, the concept of normality may be derived from statistical considerations. All human characteristics exhibit a certain range of variation. Sinclair proposes to draw the line between normal and abnormal values at ±1 standard deviation from the mean. This proposal will not be acceptable on several grounds. Most importantly, Sinclair does not specify the population represented by the sample from which the mean and standard deviation are to be derived. If the sample consists of individuals thoroughly screened with clinical methods and considered completely healthy, ±3 standard deviations is a more correct range of normality. If the sample is drawn from the "general" (ambulant) population, ±2 standard deviations appear to be the appropriate limits of normality.

II. DIETARY, BIOCHEMICAL, AND PHYSIOLOGIC METHODS FOR ASSESSMENT OF HUMAN NUTRITURE

The assessment of nutriture is considered by H. M. Sinclair (*Vitamins and Hormones* **6**, *101 (1948)*) as a diagnosis and, like the clinical diagnosis of non-nutritional diseases, it must be based on the combination of a number of disciplines. It is a job for a team of workers trained in dietetics, biochemistry, physiology, physical anthropology, and clinical medicine. A clinical examination, a dietary history, and a medical history are considered the minimum. The methods, rated as primary, secondary, and tertiary, recommended for use in rapid assessment of the nutriture in large groups, are as follows:

PRIMARY	SECONDARY	TERTIARY
Dietary		
Dietary history	Individual method of dietary assessment	
Family inventory and purchase method		
Chemical		
Hemoglobin	Pyruvate	Minerals
Erythrocrit (or erythrocyte count)	Protein with fractionation	Prothrombase clotting-time
Protein without fractionation	Ascorbic acid in leucocytes	Vitamins of the B-complex
Ascorbic acid	Vitamins of the B-complex in leucocytes	Saturation tests of water-soluble vitamins
Vitamin A and carotenoids	Phosphatase in adults	
Phosphatase in children	Fasting urinary excretion tests or load tests of water-soluble vitamins	
Functional		
	Rod threshold of dark-adapted eye	Visual acuity
		Nerve accommodation
	Endurance tests	Ergometric tests
		Psychometric tests
		Electrocardiograms
Somatometric		
Weight	Sitting height	Other somatometric determinations
Height		
Thickness of skin and subcutaneous tissue		
Clinical		
Examination of skin, eyes, etc.	Pulse rate	X-ray of hand and wrist in adults
Medical history	X-ray of hand and wrist in children	Capillary fragility
Blood pressure		Gum fragility

The main features of the five groups of methods will be discussed. For other summaries of methods for nutriture appraisal see: W. J. Dann and W. J. Darby (*Physiol. Rev.* **25,** *326 (1945)*); H. R. Sandstead and R. K. Anderson (*Pub. Health Reports* **62,** *1073 (1947)*); R. E. Johnson, F. Sargent, P. F. Robinson, and F. C. Consolazio (*War Medicine* **7,** *227 (1945)*); O. A. Bessey and O. H. Lowry (*Am. J. Pub. Health* **35,** *941 (1945)*); Lowry and Bessey (*Federation Proceedings* **4,** *268 (1943)*); and *National Research Council Bull.* **117,** *47 (1949)*.

DIETARY ASSESSMENT

A dietary history alone cannot serve, under normal circumstances, for assessment of nutriture. However, it is useful—provided the information is accurate—in interpretation of the data obtained by the direct methods for assessment of nutriture and serves as a basis for suggesting improvements in the diet when symptoms of malnutriture are found.

Methods for estimating food consumption by a country as a whole, an institution, a family (money spent, inventory of food at start and end of a week), and an individual (detailed dietary history, questionnaire estimates, measurements of food actually consumed) were noted. Except for the last method, the procedures yield too crude an estimate of per capita food consumption.

An important source of error is the estimation of food waste. This factor will be of considerably different magnitude when applied at different stages of food consumption (food as distributed wholesale, as purchased retail, as eaten). In the case of food "as eaten" one may consider the inedible part, such as bones or potato peelings, removed during preparation and left on the plate, as well as the edible part, lost during cooking and serving. Sinclair pointed out that waste factors applied in most diet tables to food "as purchased" are far too generous for areas in which there is a scarcity of food.

There are difficulties in expressing in terms of nutrients the information about food actually consumed even when the amounts of foods eaten are known precisely. These difficulties arise from such factors as variation in nutritive content of different batches of the particular foodstuff and errors in the allowances for losses in cooking. It may be added, parenthetically, that in all experimental nutritional research it is highly desirable to make direct analyses of the content of the meals eaten. However, even then there remains the thorny problem of "availability" of aliments and nutrients, complicated by losses through excretion in urine and feces as well as additions resulting from the activity of bacteria in the gut.

CHEMICAL ASSESSMENT

The chemical analysis is directed to three types of substances: nutrients and aliments (*e.g.*, blood sugar, calcium, vitamin A), constituents of the body affected by nutrition (*e.g.*, hemoglobin), and metabolic products (*e.g.*, pyruvic acid). The analysis is made on body tissues, blood, urine, and other body fluids, such as sweat and milk, and feces. Only the composition of the first three items is discussed in greater detail.

Leucocytes are considered the most satisfactory living tissue to be analyzed in nutrition surveys and are regarded as particularly valuable for study of water-soluble vitamins. Other tissues have been studied but little. Obviously, biopsies of soft or bony tissues are not easy to make, but nails and hair are collected easily and in some conditions might serve as useful indicators of nutriture. The special value of the chemical tissue analysis is indicated by the fact that the changes in tissue composition precede the changes in tissue structure.

Blood (as whole blood, plasma, or serum) and urine provide the bulk of the material for the biochemist-nutritionist. The specific methods were summarized by H. M. Sinclair ("Recent Advances in Clinical Pathology," *Ch. 18, Churchill, London (1947)*) in a chapter on biochemical aids in the diagnosis

of nutritional deficiencies. Nutritional interpretation of the findings, especially of the urinary values, is frequently anything but a straightforward matter. Urinary excretion of vitamins may serve as a good example of debatable data. The author points out that a healthy individual is not expected to excrete glucose in the urine, and wonders why he should be expected to excrete ascorbic acid. The urinary excretion of water-soluble vitamins reflects satisfactorily the immediate intake of these vitamins, but the excretion data seem to bear no clear relation to man's nutriture. The problem becomes still more intricate when determinations are made not upon fasting urine but when excretion is followed after a test dose (load test) or when saturation tests are applied.

Individual differences, not related to dietary intake, are present in the blood levels of different compounds. Why does one of 2 individuals on the same protein intake have a serum protein concentration of 6.5 g. per 100 ml., while the other one has one of 8.5 g.? Sinclair mentions body build (somatotype) as one of the factors possibly accounting for these differences but does not suggest any specific mechanisms and leaves open the question of other factors. Some blood constituents are affected by the sex and age of the subjects and these facts must be taken into account in the evaluation of data. For the effect of age and quantitative analysis of the concept of individuality see the article by A. Keys (*Federation Proceedings* **8,** *523 (1949)*).

Different compounds in plasma respond differently to changed dietary intake. The plasma concentration of carotenoids and ascorbic acid, for example, follows rapidly the alterations in intake, while protein may be within the normal range even when clinical malnutriture is present. Existence of marked individual differences is attested by the fact that a low normal value of 6.3 g. per 100 ml. may be found in individuals who had subsisted on diets grossly deficient in animal protein as well as in individuals with an adequate protein intake.

A table of arbitrary standards of normality for seventeen chemical characteristics for light threshold of the rods in dark-adapted eye, and for capillary fragility is given in the text.

FUNCTIONAL ASSESSMENT

The study of dietary intake needed for maintenance of physiologically normal functions and of deterioration of functions resulting from dietary restrictions is the central task of the quantitative science of nutrition. Sinclair discussed visual acuity, light threshold, and visual field of the dark-adapted eye, nerve irritability, capacity for physical work, cardiovascular and respiratory function, psychomotor performance, and intelligence.

A rapid failing of vision, with or without scotomata, has been reported repeatedly in warm climates as a symptom of nutritional neuropathy. It may be noted, parenthetically, that in prolonged and severe caloric undernutrition the measured visual acuity remains unaltered. The factor(s) responsible for nutritional amblyopia in man are not known, although a deficiency of some part of the vitamin B-complex is frequently incriminated. A marked increase in the rod threshold of the dark-adapted eye is an indicator of vitamin A deficiency. However, the period, within which such a phenomenon was reported to take place, varies within distressingly large limits, from a few days to many months (*Nutrition Reviews* **7,** *234 (1949)*).

To demonstrate that a high threshold is due to vitamin A deficiency, a therapeutic test is needed, because impairment of light sensitivity may be of non-nutritional origin. Determinations of changes in nerve excitability, as measured by chronaxie, in deficiency states were made by various French workers, with conflicting results. Sinclair measured nerve accommodation in 11 thiamine-restricted and 11 thiamine-supple-

mented subjects; after eighty-six days of the experimental regimen, at a time when no differentiation was possible on the basis of a clinical examination, he was able to identify correctly the nutritional status of 70 per cent of the subjects on the basis of the neurophysiologic data.

Tests of capacity for physical work and of motor functions (strength, speed, coordination) are of considerable practical interest. Sinclair noted that performance of muscular work depends on three sets of biochemical factors: the supply of aliments providing energy for work; the supply of nutrients participating in the release of energy; and the processes involved in the maintenance of homeostasis by counteracting the physicochemical changes resulting from the energy metabolism. Influence of training and motivation, likely to vitiate results if not controlled, was also duly noted. If 2 children undergo the bar test together, they will hang on the bar longer because of added motivation derived from competition; it would obviously be ridiculous to conclude that they are "better nourished" than when they hang singly. However, it is largely a contradiction of terms when the author demands that tests of endurance should involve motivation to as slight a degree as possible. What is needed is a good experimental and statistical control of the factor, not its elimination.

III. SOMATOMETRIC AND CLINICAL METHODS FOR ASSESSMENT OF HUMAN NUTRITURE

In the assessment of human nutriture an important role is assigned by H. M. Sinclair (*Vitamins and Hormones* **6,** *101 (1948)*) to clinical methods, even though the author calls for critical re-evaluation of the clinical signs of malnutriture. Somatometric methods are discussed briefly.

SOMATOMETRIC ASSESSMENT

Nutritionists have paid much attention to the growth of children as a criterion of nutriture. As a rule, a faster rate of growth was considered more desirable. The author points out that the increased rate of growth of children, observed both in the United States and in England, has not been unequivocally demonstrated as superior in terms of health. He is of the opinion that the phenomenon may actually be undesirable as the *slow* rate of maturation and long period of childhood and adolescence are typically human characteristics. He cites the work of S. B. Wolbach (*Nutrition Reviews* **3,** *193 (1945)*) on acceleration of bone growth in the rat and guinea pig fed on diets containing large amounts of vitamin A; the new bone was inadequately calcified and fractured easily. Although their relevance for human nutrition remains uncertain, the data of C. M. McCay (*Am. J. Pub. Health* **37,** *521 (1947)*) indicating that *slow* growth in the rats resulted in a much lower incidence of tumors and in a longer life span of the rat should also be considered.

The sex-age-height-weight tables are declared, somewhat brusquely, useless for characterization of nutriture in the adult. Sinclair points with justification to such extraneous factors affecting body weight as seasonal variations in nude weight, and the greater fluctuations of the clothing, and variations in the fluid content of the body, such as due to famine edema. It is well known that full health (and longevity) is compatible with a rather wide range of values of relative weight (actual weight as percentage of standard weight) and that the "standard" weight easily becomes, in the hands of the uninitiated, a fetish. However, the age-height-weight data are capable of further refinement by taking into account the bony framework of the body (H. B. Pryor, "Width-Weight

Tables," *2nd revised ed., Stanford University Press (1940)*).

In Sinclair's judgment the anthropometric indices fare no better than standard weight. In view of this it is of interest that in an investigation of the validity of different methods for assessment of nutriture, undertaken in England by R. H. Jones (*J. Roy. Statistical Soc. (London)* **101,** *1 (1938)*), experienced School Medical Officers picked out two-thirds, while the Tuxford height-weight index identified correctly three-quarters, of the pupils assessed as undernourished by two or more of the five physicians who participated in the study.

The determinations of the thickness of subcutaneous tissues (plus the skin) are mentioned as an index of fatness-thinness. The author prefers measurement of the skinfolds over the top of the biceps and midway down the back of the forearm. The studies by H. V. Meredith (*Univ. Iowa Studies Child Welfare* **12** (*No. 3*), *1 (1935)*) and B. Boynton (*Ibid.* **12** (*No. 4*), *1 (1936)*) are not referred to.

CLINICAL ASSESSMENT

This is the largest of the five sections. It is made clear from the start that clinical data are, as a rule, deficient in the repeatability of the observations when the physical examinations are made by different examiners or by the same examiner at different times, and that interpretation of them has frequently been fallacious. The accuracy of observations of some structural abnormalities is markedly increased by use of special instruments, such as an illuminated low-grade magnifier for examination of the skin and tongue, and a slit-lamp microscope for inspection of the limbus of the eye. Even when dealing with qualitative clinical signs, an objective photographic record of the lesion is a useful diagnostic aid. However, measurements should be used rather than qualitative descriptions whenever it is feasible.

Many more signs can be recorded quantitatively than are so recorded. Gingival tenderness is used as an example. In nutrition surveys a blunt probe is pressed against the gums and the tenderness rated on a gross scale, with no objective standard for the pressure applied. The author points out that a probe activated by a spring may be constructed with which the pain-threshold pressure can be measured. The physiologist routinely seeks quantitative measures and applies them in the study of normal function (such as the strength of knee jerk or vibratory sensitivity) while the clinicians in their study of the abnormal rarely apply rigorously standardized measurements.

Clinical signs of nutriture are discussed in some detail. Skin, hair, and nails; tongue, gums and teeth; mucous membranes; mucocutaneous junctions, and eyes; glands and the nervous system; connective tissue, muscles, bones, and cartilage; and respiratory, circulatory, gastrointestinal, and genitourinary systems are examined in turn. It is the critical evaluation and comment which lend the principal value to this section.

In the skin examinations the extent of the area inspected should be standardized and should be noted in the reports. It is an obvious but often forgotten fact that in a larger area there is a greater chance of finding skin abnormalities. Certain abnormalities occur with greater frequency in particular regions, *e.g.*, folliculosis on the buttocks. Another elementary but frequently overlooked fact, likely to distort statistics on the incidence of gingivitis, is that older people without teeth rarely suffer from this gum abnormality. If the fact is not taken into account, one may obtain a spurious decrement in the incidence of gingivitis with age.

Several structural abnormalities regarded as a rule as manifestation of nutritional deficiencies may result also from non-nutritional factors. Sinclair insists that their nutritional origin be adequately established before reaching dramatic conclusions about the percentages of individuals suffering from malnutrition and questions the validity of the conclusion reached in the Newfoundland survey (J. D. Adamson *et al., Canad. Med.*

Assn. J. **52,** *227 (1945)*) that gum involvement found in 69 per cent of the population examined is indicative of high incidence of ascorbic acid deficiency. Unkempt ("staring") hair; bugbites attributed to deficiency of ascorbic acid; dry scaly erythematous patches due to lack of soap; denudation of the filiform papillae of the tongue due to upper denture; redness of the fungiform papillae resulting from hot food or heavy smoking; and cracked lips and chapped skin caused by exposure are some of the lesions which are at times erroneously attributed to nutritional factors.

COMMENT

Although the point is not explicitly made by the author, rapid nutrition surveys are considered as the background against which evaluation of methods for assessment of human nutriture is made. Obviously this basic attitude will color the estimation of the value of the specific criteria of nutriture. The research worker in a laboratory engaged in experimental investigations on human nutrition or the clinician having for his primary purpose the diagnosis and treatment of nutritional deficiency diseases would consider the methods in a different light and would arrive at a somewhat different evaluation.

The emphasis on methods useful in nutrition surveys is likely to be responsible for omission of some of the more complex but fruitful methods introduced recently in nutritional research, such as estimation of body fat based on determination of specific gravity of the body (A. R. Behnke, B. G. Feen, and W. C. Welham, *J. Am. Med. Assn.* **118,** *495 (1942)*) and showing striking changes at changing levels of caloric intake (J. Brozek, *Federation Proceedings* **5,** *13 (1946)*). Similarly, the technic for determination of the thickness of skin and subcutaneous tissue, muscle, and bone from roentgenograms (H. C. Stuart, P. Hill, and C. Shaw, *Monogr. Soc. Res. Child Development* **5** *(No. 3), 1 (1940)*; E. L. Reynolds, *Child Development* **15,** *181 (1944)*) was not mentioned, although it has been applied in nutrition surveys (Stuart and D. Kuhlmann, *J. Pediatrics* **20,** *424 (1942)*).

Tests of cardiorespiratory functions, such as pulse rate after standard exercise or maximal oxygen intake, important for characterization of physical fitness, were not discussed because they were considered as being usually not practical. The exploration by modern tools of the relation between nutrition and personality (J. Brozek, H. Guetzkow, and A. Keys, *Psychosomat. Med.* **8,** *98 (1946)*) also was not mentioned.

RETROLENTAL FIBROPLASIA AND VITAMIN E

Retrolental fibroplasia is a disease which develops in some premature infants shortly after birth. The greater the prematurity, the more likely the disease is to develop. The condition is characterized by development of an active process in the retina which leads to detachment of the retina with subsequent fusion to form a membrane behind the lens. Serious visual impairment or permanent blindness results. The etiology of the disease is unknown and no effective prophylaxis or treatment has been available (*Nutrition Reviews* **7,** *169 (1949)*).

V. E. Kinsey and L. Zacharias (*J. Am. Med. Assn.* **139,** *572 (1949)*; *Nutrition Reviews, loc. cit.*) presented evidence which suggested that nutritional factors might be important in causing this disease. They found a positive correlation between the incidence of the disease and the use of relatively large amounts of supplemental iron and water-miscible vitamin preparations.

W. C. Owens and E. U. Owens (*Am. J. Ophth.* **32,** *1631 (1949)*) have reported that they were able to prevent this disease in almost every instance using large amounts of dl-alpha-tocopheryl acetate. They studied 101 premature infants weighing 3 pounds or

less at birth. A total of 63 infants had been studied before the use of tocopherol was started; 12 of these developed retrolental fibroplasia. For a period of ten months alternate infants admitted to the premature nursery were treated with the vitamin. Fifty milligrams of dl-alpha-tocopheryl acetate were given three times daily starting as soon after birth as the infants could take feedings. Both control and tocopherol-treated infants received 9000 units of vitamin A and 900 units of vitamin D daily in a water-miscible preparation. About one-third of each group received 4 ml. of 10 per cent ferric ammonium citrate daily. Fifteen infants were in the control group and 5 developed retrolental fibroplasia. Eleven infants were given tocopherol and none developed the disease. Because of these encouraging results, the plan was then changed so that all prematures weighing 3 pounds or less at birth were given tocopherol. The routine use of vitamin A and iron was discontinued although 400 units of vitamin D in water-miscible form were administered daily. Of 12 infants on this regimen only one developed the disease. Development of the disease in this case may not be a true failure since this infant had a rather stormy neonatal course, with feeding problems, and tocopherol could not be given until the eleventh day after birth.

When all the infants used in the study were considered together, a total of 78 received no tocopherol supplement. Seventeen of these (21.8 per cent) developed retrolental fibroplasia. Twenty-three infants received tocopherol and the disease was prevented in all but one (4.4 per cent).

The therapeutic value of alpha-tocopheryl acetate was studied in 9 infants showing early stages of retrolental fibroplasia. In 5 cases, progress of the disease was halted. In the other 4, the course of the disease was unaffected. These results were regarded as encouraging since spontaneous regression of the disease after the stage of diffuse retinal exudation is unusual.

In their experience, vitamin E supplements were of no value if not started at least before the baby was 6 weeks of age, since irreversible retinal changes may occur by that time. Likewise, vitamin E was of no value when the disease had progressed sufficiently to be recognized grossly by parents or physician by external examination.

Serum tocopherol levels were determined in some of the infants. Forty-six determinations from infants receiving no tocopherol supplement showed an average level of 0.25 mg. per cent. This is considerably less than the normal adult level of 1.0 to 1.2 mg. per cent. The average of one hundred and three serum tocopherol determinations in infants receiving 150 mg. of dl-alpha-tocopheryl acetate daily was 4.12 mg. per cent. In some of these infants, the serum tocopherol level did not rise even though large tocopherol supplements were given, presumably due to poor absorption. Parenteral vitamin E might be considered in such cases. No constant correlation was observed between serum tocopherol level and occurrence of retrolental fibroplasia. The one infant who developed the disease despite tocopherol administration showed an adequate serum tocopherol level.

The importance of this work from the practical standpoint is obvious since it offers a possible means of controlling this disease. The authors were conservative in interpreting their results, recognizing that no claim for a role of vitamin E in human nutrition has yet been substantiated.

Actually, it is by no means certain that the action of vitamin E in the possible prevention of this disease represents the correction of a vitamin E deficiency in the infants. Because of the large doses of tocopherol used, the high-blood levels produced, and the lack of other evidence of vitamin E deficiency, it is possible that if the vitamin is acting it is through a pharmacologic mechanism. Instances are known in animal nutrition where doses of vitamin E far above the physiologic range have definite beneficial effects. K. Schwarz (*Ann. N. Y. Acad. Sci.* **52,** *225 (1949)*) has reported that

a certain type of dietary liver injury is not prevented by the usually sufficient dosage of tocopherol, but when the amount of the vitamin was increased to seventeen times the normal dose, liver degeneration was completely prevented.

Although the requirement of the human organism for vitamin E is not known, it would be surprising if a vitamin which is active in such small amounts in animals would be required in amounts of 150 mg. per day by a 3-pound infant. Owens and Owens have not used smaller amounts of vitamin E and so it cannot be stated whether smaller doses of the vitamin might be effective.

Several facts are known which might lead one to suspect that the premature infant is deficient in vitamin E, as pointed out by Owens and Owens. The low-fat diet customarily given prematures is very low in vitamin E and none is routinely given as a supplement. Apparently there is very little placental transfer of this vitamin so that the infant may start life with very small stores. There is some evidence that immature animals are unusually susceptible to vitamin E deficiency. Prematures absorb fat-soluble substances poorly. Fat absorption itself is limited and abnormal. Unsaturated fatty acids appear to be absorbed most readily. Large amounts of vitamin A and unsaturated fats have been reported to increase the requirement of animals for vitamin E. The more or less routine use of iron supplements in prematures might further reduce the vitamin E available, since ferric salts destroy this vitamin. Other additional interesting facts about the metabolism of prematures which might influence the vitamin E requirement are included in the paper.

AMINO ACID REQUIREMENTS OF MAN

The long and careful series of investigations by W. C. Rose and co-workers dealing with essential amino acids for rats are well known. These investigations led to the discovery, isolation, and synthesis of the amino acid, threonine. Subsequently, valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophan, lysine, and histidine were shown to be essential for growth in the rat. Elimination of any one of these amino acids from the diet led to profound and rapid nutritive failure, and eventually death. Arginine was found to be necessary for a maximal rate of growth of rats fed the above mixture of amino acids, but nutritive failure did not follow omission of it from the diet. The conclusion was drawn that arginine was synthesized by the rat, but not at a rate sufficient to permit a maximal rate of gain.

Since the nonessential amino acids are present in proteins of rat tissue, it was evident that these nonessential amino acids, in contrast to those of the above list, were readily synthesized by the rat. Subsequently, it has been shown that with rations which permit a faster growth rate, synthesis of certain of these "nonessential" amino acids (*e.g.*, glutamic acid) may become the growth-limiting reaction. Under these conditions, addition of such a "nonessential" amino acid will increase the growth rate (*Nutrition Reviews* **8,** *9 (1950)*).

Through establishment of the amino acid requirements of rats, an efficient approach to the study of the amino acid requirements of man has been made possible. Results of such a study, which has been in progress since 1942, are summarized by W. C. Rose (*Federation Proceedings* **8,** *546 (1949)*).

Experimental subjects were healthy male graduate students who received a diet composed of corn starch, sucrose, protein-free butterfat, corn oil, inorganic salts, and vitamins, supplemented with an appropriate mixture of amino acids. The total nitrogen intake on such diets was 6.7 to 10 g. per

day; of this total, only 0.3 to 0.35 g. was of unknown nature. Sufficiency or insufficiency of the dietary regimen was established by the nitrogen balance technic. The qualitative results were clearcut: to maintain nitrogen balance in these adult men, only eight amino acids were required. These were valine, leucine, isoleucine, threonine, methionine, lysine, phenylalanine, and tryptophan. Arginine and histidine were not required. This result with arginine was not unexpected, since the rat also synthesizes sufficient arginine to maintain nitrogen balance under similar conditions. The result with histidine, however, was unexpected, and represents a distinct difference between the requirements of rats and those of man. The rat cannot synthesize this amino acid, whereas man can. In view of this distinct difference in requirements, the large area of agreement should perhaps be emphasized. Eight of the nine amino acids required by the rat are also required by man, while all of the amino acids which the rat synthesizes are also synthesized by man.

Having established qualitatively the amino acid requirements of man, a further study to establish the quantitative requirements was initiated. Such a study is replete with experimental difficulties which were fully recognized and considered by the author. To eliminate many of these difficulties a distinctly negative nitrogen balance was induced during experiments with any given amino acid by feeding it at progressively lower levels. The level at which it was fed was then increased until a consistently positive balance was attained over an interval of several days. The caloric content of all such diets was maintained at a level sufficient to maintain positive nitrogen balance when the complete mixture of amino acids was fed. The minimal daily requirements for a given amino acid were considered to be the highest amount found necessary to keep any of the experimental subjects in nitrogen balance. These minimal requirements were found to be, in grams per day: L-tryptophan, 0.25; L-phenylalanine, 1.1; L-lysine, 0.80; L-threonine, 0.50; L-valine, 0.80; L-methionine, 1.1; L-leucine, 1.1; and L-isoleucine, 0.70. From 8 to 31 subjects were tested in establishing each of these minimal levels.

Significant differences in the amino acid requirements of individuals were found. Perhaps the most striking instance of this was in the requirement for L-tryptophan. For 2 individuals, a minimum requirement of 0.15 g. per day was found, and 11 other subjects whose minimal requirements were not determined were kept in nitrogen balance with intakes of 0.20 g. per day. However, one individual was found to require 0.25 g. per day to maintain nitrogen balance. Because of this variation, it was deemed advisable to set the recommended daily intake at twice the value found for the minimum daily requirement. Since the latter value is the highest of those found experimentally, the recommended daily intake is thought to allow an adequate margin of safety.

During these quantitative experiments, the total nitrogen content of the experimental diets was maintained constant by supplying an amount of glycine or urea equivalent in nitrogen content to that of the omitted amino acid. This was necessary to insure an adequate supply of nitrogen for synthesis of the nonessential amino acids. Both glycine and urea can be thus used in rats. It is apparent that if minimal amounts of each of these essential amino acids were supplied, it would be necessary to increase significantly the supply of nitrogen for such synthesis. The minimal requirement of nitrogen for this purpose, or the form in which it is best supplied, are subjects for future investigation.

Some information concerning the utility of D-amino acids to man was obtained. For maintenance of nitrogen equilibrium in man, D- and L-methionine were found equally effective, and D-phenylalanine showed partial activity when substituted for L-phenylalanine. The D-isomers of valine, leucine, iso-

leucine, threonine, lysine, and tryptophan were apparently not available to any significant extent. The finding with D-tryptophan is again opposed to that in the rat, for which this amino acid shows significant activity. Acetyl-D-tryptophan also was not available as a source of tryptophan in man.

These latter findings are in essential agreement with those of H. R. Baldwin and C. P. Berg (*J. Nutrition* **39,** *203 (1949)*) who independently assessed the minimal requirement of man for L-tryptophan at about 0.20 g. per day, and found that D-tryptophan showed slight, if any, activity, and that acetyl-D-tryptophan was inactive. Conclusions from these carefully conducted investigations are at variance with earlier reports (A. A. Albanese, J. E. Frankston, and V. Irby, *J. Biol. Chem.* **160,** *31 (1945)*; L. E. Holt, Albanese, Frankston, and Irby, *Bull. Johns Hopkins Hosp.* **75,** *353 (1944)*) which held that acetyl-D-tryptophan was available to man, and that the daily requirement for tryptophan in man was of the order of 6.0 to 9.0 mg. per kilogram (420 to 630 mg. for a man weighing 70 kg.) per day.

One of the most surprising findings to come out of this investigation is that the caloric requirements necessary to achieve nitrogen balance are significantly higher (about 45 calories per kilogram) when amino acids or protein hydrolysates are fed, than when intact protein (casein, about 35 calories per kilogram) is fed. Such a difference implies operation of some factor of basic importance in nutrition, investigation of which will now become possible. Similarly, assessment of these requirements will permit attack on a great many additional problems, such as the relative requirements of methionine for protein synthesis and for methylation, the sparing action of carbohydrates on amino acids, etc., all of which are of great importance to the complete understanding of human nutrition.

FOOD INTAKE AND BLOOD CONTENT OF ASCORBIC ACID

Previous reviews (*Nutrition Reviews* **3,** *106, 210 (1945)*; **4,** *255, 347 (1946)*) have considered the findings of nutrition surveys in Wayne and Alamance Counties, North Carolina, and among Otomi Indians in the Mezquital Valley of Mexico. These studies were made during 1942 to 1944 and included seven-day diet records and laboratory and clinical assessments. The methods employed in the three surveys were comparable. There has now appeared an informative report of a statistical study of certain of the data from these three surveys (P. Putnam *et al.*, *Milbank Memorial Fund Quart.* **27,** *355 (1949)*).

The present analysis concerns data provided by the diet records and the laboratory tests. For the study the data from each area were divided into the categories of children under 15 years of age, adult males, and adult females. The study of the correlations between fourteen pairs of nutrients in the foods consumed indicated "significant correlation of a fairly high order." Similar, but less definite, correlations were observed to exist between the blood content of several factors studied, particularly between ascorbic acid and carotene. The correlations between dietary intake of a nutrient and the blood concentration of the same substance revealed that a consistently significant correlation existed only in the case of ascorbic acid intake and plasma or serum level. Since the level of ascorbic acid in the blood has been demonstrated to rise or fall promptly with corresponding variations in intake, such observations were not unexpected if the dietary record employed possessed any degree of reliability.

This report may well serve as an illustration of the methods and type of findings to be expected when survey data are examined

for the existence, degree, and form of association between intake and blood levels of a nutrient. Certain characteristics of the populations are worthy of recall: In North Carolina the intakes and plasma levels of ascorbic acid tended to be highest in summer and lower in the winter and spring, and the values of plasma levels for children were higher than for adults. It was during these latter seasons (winter and spring) that significant correlations between intake and blood levels were most often encountered. The mean plasma ascorbic acid levels for adults in North Carolina ranged from 0.43 to 0.59 mg. per cent, in contrast to the concentration of 1.2 mg. for adult Otomi Indians. In North Carolina the children had higher average plasma levels (0.76 and 0.87 mg.) than the adults, while there was no difference between the Indian children and Indian adults. A large portion of the ascorbic acid ingested by the Mexican Indians was derived from the fermented beverage, pulque. The alcohol from this drink constituted up to 15 per cent of the total caloric intake. Accordingly, there existed a rather frequently occurring significant association between ascorbic acid intake and calories, an association which was much less pronounced in the North Carolina populations.

None of the population groups was homogeneous; for example, there was no elimination from the sample of pregnant or lactating women. The correlation coefficients of intake and plasma concentration, while often indicating significant association between intake and blood level, were of rather low order—ranging from 0.28 to 0.738. The highest coefficients were obtained when sex, age, and season were simultaneously considered. Consistently low coefficients (0.110 to 0.218) were noted among the Otomi Indians whose mean plasma levels were approximately 1.2 mg. per cent. These facts are most likely related to the inhomogeneity of the population samples (*e.g.*, inclusion of pregnant and lactating women in the sample), to the exclusion from the intake estimates of ascorbic acid from cooked foods (for North Carolina), to the limitations of the dietary record as a technic, and to the exceedingly high-serum levels of the Otomi Indians—serum levels corresponding to the renal threshold for ascorbic acid.

A fourfold table analysis employing chi-square of the relationship indicated that for each North Carolina group a significantly greater proportion of persons with low-ascorbic acid intakes fell into the low-blood content group and vice versa. Hence, there was additional evidence of the existence of an association between intake and blood level. A study of the "goodness of fit" of the regression equation for plasma ascorbic acid and intake revealed that low-serum levels may be expected more frequently among persons with low intakes. This has long been a tacit assumption by those making nutrition surveys, but it has seldom been clearly demonstrated.

In an effort to define more clearly the nature of these two relationships in population groups, it was noted that the asymmetric distribution of intakes with peaks to the left of center could be converted into "normal curves" of distribution by conversion to logarithms of the intake values. Studies are reported of the relationship between log of intake in terms of blood level and vice versa. The extension of this study technic reminds one of the log-dose response test widely employed in pharmacology. It did not seem to add greatly to the treatment of the data in hand, except to provide a convenient straight-line treatment. Further exploration of this treatment seems justified, however.

Two additional points worthy of comment were found in this study: (1) Among the adult Indians blood levels of 1.12 mg. of ascorbic acid were maintained by estimated intakes of 32 mg. per day, while in North Carolina the plasma content fell below this amount on intakes as high as 147 mg. per day. "Obviously the recorded intake figures do not tell the entire story about ascorbic

acid nutriture." (2) Among the Indians, body weight was positively associated with total calories and with log of ascorbic acid intake.

So that the usefulness and limitations of survey methods may be defined more clearly, it is hoped that additional reports of this kind may be made from time to time.

VITAMIN A AND VISION

Vision in dim light (scotopic vision) is dependent on the normal functioning of the retinal rods. There is no sensation of color when light falls on rods and the spectral distribution of sensitivity in scotopic vision in most animal species can be interpreted in terms of a single pigment in the rods, either rhodopsin or porphyropsin (*Nutrition Reviews* **4,** *67 (1946)*).

Rhodopsin, or visual purple, is a conjugated protein in which the prosthetic group appears to be derived from vitamin A_1 or its aldehyde, retinene$_1$ (*Nutrition Reviews* **6,** *63 (1948)*). Regeneration of rhodopsin, which is bleached by bright light, requires the presence of vitamin A_1. Night blindness is the common term for the defect in vision in dim light that characterizes vitamin A deficiency.

Despite clearcut evidence of the importance of A_1 in scotopic vision, the exact chemical role of the vitamin and of its aldehyde, retinene$_1$, and the relation of the two substances to rhodopsin are not known for certain. The absorption maxima of A_1, retinene$_1$, and rhodopsin are 328, 370 to 390, and 500 mμ, respectively. If, as is generally believed, rhodopsin is formed from A_1 or retinene$_1$, an explanation must be found for displacement of the absorption spectrum of these possible precursors to the 500-mμ band of visual purple (*Nutrition Reviews* **7,** *339 (1949)*). Decomposition of the pigment of the rods is known to yield indicator or visual yellow which shows a maximum of 440 mμ in acidic solution and of 365 mμ in alkaline solution. No explanation has been found for the sensitivity of indicator yellow to changes in hydrogen ion concentration.

This problem is discussed by S. Ball, F. D. Collins, P. D. Dalvi, and R. A. Morton (*Biochem. J.* **45,** *304 (1949)*) who report that the characteristic absorption spectrums of indicator yellow in acidic and basic solutions were duplicated by products formed *in vitro* from retinene$_1$ and various nitrogenous compounds. When retinene$_1$ was allowed to stand for a short time in alkaline solution with an excess of an amino acid or of a protein, the solution exhibited the absorption spectrum of alkaline-indicator yellow and, on acidification, that of acid-indicator yellow. The data suggested that conjugated proteins resembling and including indicator yellow possess two retinene$_1$ molecules attached to the same nitrogen atom of the protein. On the other hand, retinene$_1$, treated in acidic solution with benzylaniline and similar compounds, gave red solutions with absorption maxima of about 500 mμ. This showed that retinene$_1$ can give rise to a rhodopsin-like chromophore.

Although it was not clear what structural linkages accounted for a product similar to indicator yellow in one instance and to a product similar to rhodopsin under other conditions, the data are important because of the demonstration that some retinene derivatives actually resemble rhodopsin and indicator yellow. It should be noted, however, that the possibility remains that rhodopsin may be a derivative of A_1 rather than of retinene$_1$.

Vision in bright light (photopic vision) and the accompanying differentiation of colors are related to the retinal cones. The phenomenon of color sensation cannot be explained on the basis of a receptor compound with a single absorption maximum. In this connection, R. Granit ("Sensory

Mechanisms of the Retina," *Oxford University Press, London (1947)*) has postulated a dominator-modulator theory of photopic vision in which the dominator is concerned with the sensation of brightness and the modulator with the perception of color. The data accumulated by Granit show that the action of the modulator involves absorption in three spectral regions, 440 to 470, 520 to 540, and 580 to 600 mμ. Little is known of compounds in the cones that may be responsible for these specific absorption spectrums. G. Wald (*Nature* **140,** *545 (1937)*) obtained evidence for a cone pigment, iodopsin, in chicken retinas with an absorption maximum of 575 mμ.

The possibility that vitamin A may be involved in photopic as well as scotopic vision should be given serious consideration according to S. Ball and R. A. Morton (*Biochem. J.* **45,** *298 (1949)*). These investigators demonstrated that products with absorption spectrums corresponding closely to the three maxima postulated by Granit are formed from vitamin A_1 and from retinene$_1$ by the action of cold concentrated acids. The compounds were believed to belong to the class of "ionized" or "halochromic" molecules described by P. Meunier and A. Vinet ("Chromatographie et Mesomerie," *Masson et Cie, Paris (1947)*) as products of the reaction between vitamin A and antimony trichloride. The variations in absorption spectrums were considered due to polarization of the vitamin A molecule and a formation of strongly resonating structures in the conjugated double-bond system of the vitamin.

Ball and Morton concluded that photopic receptor substances, presumably conjugated proteins with A_1 or a derivative as the prosthetic group, may be present in retinal cones in the form of halochromic particles that account for the modulator effect. These authors emphasized the unphysiologic nature of the chemical procedure which yielded the derivatives of A_1 showing the required spectrums. Nevertheless, suitable enzymes might readily bring about the same reactions under physiologic conditions and their results strengthen the possibility that the vitamin A_1-retinene$_1$ system in retinal cones functions in vision in bright light.

The studies by Morton *et al.* do not demonstrate conclusively that vitamin A and its derivatives are the sole pigments in rods and cones responsible for vision in both dim and bright light. The findings are highly suggestive nevertheless. The difficulty in understanding the lack of apparent connection between rhodopsin and vitamin A_1, based on difference in absorption spectrums, would appear to be removed by the demonstration that retinene$_1$ does form addition products in which a similar shift in the absorption spectrum occurs.

The hypothesis that visual purple is a riboflavin complex is no longer necessary although riboflavin may be involved in the coupled reaction in which retinene is formed from A (*Nutrition Reviews* **7,** *339 (1949)*). The additional finding that A_1 and retinene$_1$ form products showing the absorption maxima which seem to be necessary for color perception agrees with the observations of G. Wald, H. Jeghers, and J. Arminio (*Am. J. Physiol.* **123,** *732 (1938)*) and of S. Hecht and J. Mandelbaum (*Science* **88,** *219 (1938)*). Both groups of workers noted similar changes in rods and cones in human subjects on A-deficient and A-supplemented diets and concluded that vitamin A is the precursor of photoreceptor pigments in the cones as well as in the rods of the retina.

NUTRITIONAL STATUS AND OSSIFICATION OF THE BONES OF CHILDREN

Ossification of the bones of the body begins as an orderly process *in utero* and proceeds through the first two decades of life. The deposition of bone is initiated in centers of ossification which appear at specific times. Bones of the hand and the epiphyses of the distal portions of the radius and ulna ossify from centers appearing in orderly fashion from soon after birth until about the sixth year. Roentgenographic evidence has accumulated demonstrating relative uniformity in time and sequence of appearance of these centers of ossification in normal children (C. C. Francis and P. P. Werle, *Am. J. Phys. Anthropol.* **24,** *273 (1939)*; J. W. Pryor, *Kentucky Med. J.* **13,** *481 (1915)*).

Illness has been shown to cause delay or interruption of ossification. It has been claimed that the appearance and rate of ossification of these centers relates importantly to the state of nutrition of the child (T. W. Todd, "Atlas of Skeletal Maturation: I. The Hand," *C. V. Mosby Co., St. Louis (1937)*).

Osseous development of the bones of the hand and wrist has been studied in a group of undernourished schoolchildren in Florida (O. D. Abbott, R. O. Townsend, R. B. French, and C. F. Ahmann, *Am. J. Dis. Child.* **79,** *69 (1950)*). The children included in this group were from a school in a poor rural area and from homes which were economically unstable. Diets were poor and there was great prevalence of physical signs of nutritional deficiencies. Earlier observations, by these same investigators, on this group of children have been published (*Florida Agr. Exp. Sta. Bull.* **426** *(1946)*; *Nutrition Reviews* **7,** *101 (1949)*).

Roentgenograms were made of the right hand of each of 162 children aged 6 to 13 years. The chronologic age of each child was recorded and compared with the bone age as determined by comparison of the roentgenograms with descriptive guides and observed times of appearance of centers of ossification in the bones of the hands of normal children. Seven carpal bones and the distal epiphyses of the radius and ulna were included in the bones studied.

Data accumulated indicated retardation in carpal development. This was most marked in boys 6 to 9 years of age and in girls aged 8 and 10 years. The carpal age as determined in this investigation was 26 months less than the chronologic age in 10-year-old girls and 24 months less in girls of 8 years. The average retardation in carpal growth was 27, 25, and 25 months, respectively, in 6-, 7-, and 8-year-old boys.

In each roentgenogram the development of each carpal bone and two epiphyses was assessed and expressed as mature, immature, or missing. The three oldest carpal bones, the capitate, hamate, and triquetrum, begin ossification during the first year of life. Among the girls of the schoolchildren studied these bones were classified as immature in about 65 per cent at age 7, and 33 per cent at age 11. In the boys, retardation in development of these bones was most marked at 6 years when about 80 per cent were shown to be immature. Seventeen per cent of boys 12 years of age had delayed ossification or immaturity of these carpal bones.

The lunate is the carpal bone next in sequence of development. There was marked variation in the time of appearance and degree of development of this bone.

Three carpal bones, the greater multangular, navicular, and lesser multangular, normally begin ossification during the third year of life in girls and during the fourth year in boys. These bones were missing or immature in a large majority of the children of both sexes in the age group 6 to 9 years. The data also indicated that if the normal sequence of appearance of centers of ossification was interrupted, the center for the bone affected may be delayed until all other carpal centers

are laid down. However, maturity is quickly achieved in the bones so delayed in ossification.

The center of ossification in the distal epiphysis of the radius is due to appear at about the sixth month of life. Percentages of girls demonstrating immaturity of this bone varied from 9 to 73 per cent in the different age groups. There was a similar high proportion of boys of all ages with evidence of immaturity of this epiphysis.

The epiphysis at the distal end of the ulna of girls normally begins ossification during the fourth year. However, 53 per cent of girls of 6 years, 32 per cent of those 7 years of age, and 27 per cent of the 8-year-old group were found to have this center missing. The percentages in boys of the same age groups were 88, 46, and 28 per cent respectively. In boys, the center is due to appear during the sixth year of life. This epiphysis was present in all of the children by the ninth year but evidences of immaturity of this bone persisted in many of the children until the eleventh year.

The roentgenologic observations of Abbott and associates (*loc. cit.*) demonstrate marked retardation in ossification of the bones of the hand in this group of undernourished children. Numerous earlier reports have indicated that there is some variation in the time of appearance of these centers. It can perhaps be reasonably stated that a delay in ossification in a given center greater than twelve months is one without the realm of normal variation. In large proportions of the Florida schoolchildren retardation exceeded this period.

One must conclude that the retardation observed in this group was significant and important. Since the children were on diets demonstrated to be grossly inadequate, it is likely that malnutrition was an important factor in producing this delay in ossification.

Evidence is presented that the deficiency is not one of vitamin D, but more likely one of calcium and phosphorus, since consumption of milk was exceedingly small. It should be pointed out that infections and other diseases occurred frequently in this group of children and may have contributed importantly to the interruption of ossification. Nevertheless, malnutrition would seem to be the most important etiologic factor.

Roentgenologic appraisal of the bony development of the hand in children would seem to offer real opportunities for addition of another index of nutritional status. It is anticipated that the method will be utilized by other workers and will be further defined and evaluated as a tool in nutritional appraisal.

IRON DEFICIENCY IN WEANLING RATS

Iron deficiency (hypoferric) anemia in infancy continues to be an important preventable medical problem. G. M. Guest ("Symposia on Nutrition of the Robert Gould Foundation," *Vol. I, p. 144, Robert Gould Foundation, Inc., Cincinnati (1947)*) has shown that lesser degrees of the condition are not uncommon and that there is a tendency for an increasing degree of microcytosis to develop in successive siblings born to the same mother. Guest found microcytosis to be a more sensitive index of iron deficiency in infants than hemoglobin concentration alone. This microcytosis could be prevented or cured by administration of iron.

Considerable attention has been directed at efforts to assign the relative importance of maternal iron deficiency and, hence, low-iron stores of the newborn and of the postnatal diet in the pathogenesis of hypoferric anemia of infants. M. B. Strauss (*J. Clin. Invest.* **12,** *345 (1933)*) emphasized the association of iron deficiency anemia in mothers and infants. Some other workers have doubted that this association indicated a cause and effect relation (L. S. P. Davidson

and H. W. Fullerton, *Edinburgh Med. J.* **45,** *1 (1938)*). Whatever may be the factors involved, it does appear that children in some families have a tendency to become iron deficient.

A report which may contain observations bearing on this problem is that of A. L. Bacharach, W. F. J. Cuthbertson, and D. M. Thornton (*Brit. J. Nutrition* **3,** *3 (1949)*) which describes an outbreak of iron deficiency anemia in weanling stock rats. In the preparation of rats for bioassay of vitamin A, the workers made a practice of replacing the stock diet available to mothers and their young with a vitamin A-free diet when the pups were 10 days old. This vitamin A-free diet contained salts of the usual type and provided 32 parts per million of iron. Animals were weaned at 24 days of age.

The stock diet originally contained whole wheat flour as its chief source of iron. This was changed to National flour of some 85 per cent extraction, and then in May, 1947, to Canadian "D" flour of 70 to 72 per cent extraction due to regulations promulgated by the Ministry of Food. This modified stock diet contained 16.4 p.p.m. of iron. In July, 1947, it was observed that the weanling albino rats which had been prepared for vitamin A assay appeared healthier (larger, and had pinker eyes, ears, and tails) than did weanling rats in the colony which had been kept throughout on the stock diet. Red cell counts of the weanlings revealed that those which had received the vitamin A-free diet had 3.5 to 4.0 million cells per cubic millimeter, while corresponding animals fed the stock diet throughout had 1.5 to 2.8 million per cubic millimeter. Cell size was stated to be normal. The healthy weanling rat has a count of approximately 5.0 million (M. V. Gardner, *J. Franklin Institute* **243,** *77 (1947)*). If this observed difference was indeed due to the difference in iron content of the two diets during the fourteen days immediately pre-weaning, it is apparent that transmission of iron through the placenta or mammary gland of the mother would be of minor importance in its prevention compared with the diet directly consumed by the pups. On the other hand, it may have been that a reduced intake of iron by the mother resulted in such low stores of the element at birth that the deficiency could readily become manifest. It would have been useful if measurements of tissue (especially liver) iron content had been made at birth in these animals and in the newborn of mothers receiving the same diet supplemented with iron.

Perhaps the most interesting observation related to this outbreak of anemia in rats was that it appeared in only one of two strains of rats which were maintained in the colony on the identical stock regimen. Thus, in September, 1947, weanlings of strain WAG had erythrocyte counts ranging from 1.70 to 2.59 million while those of strain PVG ranged from 6.06 to 6.54 million. The young of these two strains were comparable in weight. It was suggested, therefore, that this greater susceptibility to nutritional hypochromic anemia may be an expression of an interstrain difference in the metabolism of iron. If so, the study of the metabolic economy of iron in the two strains should yield information of fundamental importance. Whether it would bear on the tendency of infants of some mothers to develop hypoferric anemia is conjectural, but worthy of attention.

This report by Bacharach *et al.* records only the red cell counts of the animals under observation. Inclusion of data on hemoglobin and packed cell volumes would have aided greatly in appraising the validity of the conclusions of the investigators. Erythrocyte counts alone are generally of limited value in the study of iron deficiency, inasmuch as the total number of red cells is altered less than are the hemoglobin concentration and the packed cell volumes. Further, the sampling error of erythrocyte counts is greater than is that of the determination of either hemoglobin or packed cell volume.

NOTES

Letter to the Editor

Dear Sir:

We were much interested in your review on the "Role of Tryptophan in the Biosynthesis of Niacin" (*Nutrition Reviews* **7,** *307 (1949)*). The possibility of a catalytic function for tryptophan in the synthesis of nicotinamide is an interesting one. However, it was with some surprise that we failed to find any mention of our work (C. Heidelberger, E. P. Abraham, and S. Lepkovsky, *J. Biol. Chem.* **176,** *1461 (1948)*; *Ibid.* **179,** *151 (1949)*). These articles, one of which appeared considerably before the paper referred to most extensively in your review, show by means of isotopic experiments that tryptophan *is* converted directly into nicotinamide. This work with the intact animal, together with that of other laboratories that used enzyme systems, appears to present conclusive evidence in support of the direct conversion of tryptophan into nicotinamide. (*Note:* This letter was received *prior* to the review "Conversion of Tryptophan to Niacin Demonstrated by Radiocarbon," *Nutrition Reviews* **8,** *85 (1950)*).

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Nutrition and Industrial Poisons

The relation between diet consumed and poisoning from industrial compounds, particularly benzene and the nitrobenzene substances, unfortunately has been derived largely from uncontrolled clinical and laboratory studies. This misfortune has been stressed in previous reviews (*Nutrition Reviews* **1,** *92, 425 (1943)*; **6,** *215, 289 (1948)*).

In an effort to bring some order out of the chaos, the Food and Nutrition Board of the National Research Council appointed a subcommittee on the relation of nutrition to toxic reactions in industry (*Nutrition Reviews* **3,** *352 (1945)*). M. E. Shils and L. J. Goldwater (*J. Indust. Hyg. Toxicol.* **31,** *175 (1949)*) have prepared an article, at the request of this subcommittee, concerning the nutritional factors affecting the toxicity of certain hydrocarbons, particularly the benzene and nitrobenzene compounds. The review is a critical one and again emphasizes the lack of control in many of the clinical and experimental studies which have been made.

Although the conclusions of the authors do not represent necessarily the opinions or recommendations of the National Research Council, they appear to be based upon sound judgment. With respect to trinitrotoluene (TNT) and related compounds, the authors conclude that a deficiency of protein with or without large amounts of fat in the diet predisposes to damage of the liver. The same is true of ascorbic acid deficiency. Despite this, milk and ascorbic acid have not been shown to be specific prophylactics against TNT poisoning.

The best evidence would appear to indicate that well-balanced meals containing adequate protein and moderately low in fat should be recommended for workers exposed to the benzene and nitrobenzene compounds.

As the authors note, further studies are necessary, both in man and animals, to clarify many of the points concerning the relation of nutrition to industrial poisons. Nevertheless, it is encouraging to find that on the basis of present evidence no marked alteration from a simple nutritious diet need be recommended. It should be emphasized, however, that workers in industries employing these poisons be given some information about the composition of a nutritious diet. The continued need for nutrition education in industry is clear.

WAC and WAF Calories

Problems connected with plans to reduce the daily ration content for personnel of the Women's Army Corps and Women in the Air Force to a minimum of 2400 calories are being studied.

The standard ration for both Army and Air Force has provided for a minimum of 3600 calories per person per day, regardless of sex.

Studies show that WAC and WAF personnel have a tendency to gain weight on the 3600-calorie diet designed for men. This often necessitates issue of larger uniforms a few months after enlistment and involves problems of physical fitness. Plans for reducing the caloric content for female personnel do not necessarily imply lessening the total amounts of food, it is pointed out, but rather to adapting better the menus to fit women's food preferences on a lower caloric level.

Change No. 1, to Army Regulation 40-250, published in February 1949, provides for changing the basic standards of diet for female personnel. Under the 2400-calorie level for female personnel, the nutrient intake is designated to include: protein, 60 g.; calcium, 700 mg.; vitamin A, 5000 I.U.; thiamine, 1.5 mg.; riboflavin, 1.5 mg.; niacin, 15 mg.; and vitamin C, 50 mg. Although these standards were established by the regulation, methods for implementing them were left to future research and experimentation.

Use of Pyridoxine and Adrenal Cortical Extract in Nausea and Vomiting of Pregnancy

Innumerable methods have been proposed to control the nausea and vomiting which occurs so frequently during the first trimester of pregnancy. Only a few of these have found a secure place in the physician's armamentarium and none is completely satisfactory. Since the etiology of the condition is not known, treatment has of necessity been empirical. Pyridoxine has been advocated for this disease and has found favor with many obstetricians despite the fact that proof of its value is debatable.

C. M. Dorsey (*Am. J. Obstet. Gynec.* **58,** *1073 (1949)*) reported that injections of pyridoxine combined with adrenal cortex extract produce excellent results in this condition, results apparently superior to that expected from either substance alone. Twenty-five milligrams of pyridoxine were combined with 0.5 ml. of adrenal cortex extract and given intramuscularly to patients who were resistant to ordinary supportive therapy (bed rest, mild sedation, repeated small feedings). One, two, or rarely as many as ten injections were given at one to five-day intervals. The preferred routine was two injections twenty-four hours apart, additional injections being given when and if symptoms returned. Supportive therapy was continued during the injections. No controls were used nor were the results compared with any other system of therapy.

Sixty-two patients, 30 primiparas and 32 multiparas, were treated. Of these, 56 were classified as complete cures. Two of 3 others showed such marked improvement that they were considered as successful results so that the percentage of cure approached 95 per cent. The 3 failures had other complications of pregnancy which may have interfered with their response.

This report must be treated with considerable conservatism. The tremendous influence exerted by psychic factors in this condition is well known. The lack of controls, the lack of data as to the severity of the average patient treated, and the tendency of the usual patient to cure spontaneously during the third or fourth month of pregnancy make it impossible to evaluate this report critically.

Nevertheless, any report claiming results so definitely superior to those usually obtained by obstetricians cannot be dismissed without serious consideration. If the system is equally successful in the hands of others, a substantial contribution will have been made in the treatment of a troublesome and frequently dangerous condition.